Indian Council of Medical Research



TECHNICAL REPORT

OF THE

SCIENTIFIC ADVISORY BOARD

FOR THE YEAR

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III. TECHNICAL REPORT OF THE RESEARCHES CARRIED OUT DURING THE YEAR 1953.

The researches carried out during the year under report were recommended by the Scientific Advisory Board at its meetings held in Jaipur on the 3rd and 4th December, 1952 and were approved by the Governing Body of the Indian Council of Medical Research at its meeting held in New Delhi on the 28th March, 1953. *The views expressed by the individual workers are not necessarily the views of the Council.*

CHOLERA

1. Inquiry on the immunological studies of Vibrio-choleræ under the Director, Central Research Institute, Kasauli.

The purpose of this inquiry is to study questions relating to the immunizing efficiency of cholera vaccines with particular reference to the establishment of international reference preparations for the assay of the potency of cholera vaccine.

At the request of the Biological Standardization Committee of the W.H.O., tests were carried out on the comparative protective value of different samples of cholera vaccines of unknown origin received from the State Serum Institute, Copenhagen. The methods used for the evaluation of the potency of these vaccines were :—

1. Active immunization } of mice followed by test infection with
2. Passive immunization } a virulent strain of *V. choleræ*.
3. Assessment of vibriocidal titre after immunization with two doses of vaccine.
4. Serological examination of vaccines using mono-specific Ogawa, Inaba and cholera rough 'O' sera.

The results of tests brought out the following interesting facts (*see* ...bles I to IV) :—

Two vaccines (Nos. 144 and 145) out of the eight tested can be weeded ...as unsatisfactory by serological examination alone, provided cholera ...h 'O' serum is employed for the purpose. Vaccine No 144 has been ...ared from a strain which is in the smooth-rough phase and vaccine ...145 has been prepared from a strain which is almost entirely rough. ...e vaccines evoke poor vibriocidal response in guinea-pigs and afford ...protection to mice as evidenced by active and passive immunity tests ...ther six vaccines, Nos. 141, 142, 143, 147, 73849 and 73850, appear ...e been prepared from smooth strains of *V. choleræ*; they pass the ...ical tests, evoke good vibriocidal response and afford considerable ...ion to mice. In our opinion, these vaccines are satisfactory products ...ctical purposes. It is interesting to note that vaccines which evoke ...ibriocidal response, Nos. 144, 145, are also poor in affording ...ive, and (*b*) active immunity to mice and further that such ...can be rejected as unsatisfactory by serological tests involving ...of cholera rough 'O' serum.

Table I.

Active protection test of cholera vaccines.

Experimental animals, Swiss mice, 17 g. to 22 g. in weight.

Challenge dose at two different test levels, 10^{-3} and 10^{-4} given intraperitoneally 10 days after second immunizing dose.

Period of observation, 72 hours.

Immunizing dose, two doses of vaccine at weekly intervals subcutaneously.

Total volume of challenge dose equal to 0·5 c.c. in 5 per cent mucin'ed ... peptone.

Ogawa vaccines.

Vaccine number	Challenge strain	Challenge dose	Total of two doses of vaccine injected, c.c. 0·16	0·04	0·01	0·0025	0·000625	0·00015625	Controls, ...		Viable count
142	V. cholerae subtype Ogawa strain 423/52	10^{-3}	3/6	3/6	4/6	1/6	2/6	2/6	10^{-2}	0/6	15,160,000
		10^{-4}	3/4	5/5	6/6	6/6	6/6	4/6	10^{-3}	0/6	1,516,000
144		10^{-3}	2/6	2/6	1/6	0/6	0/6	0/6	10^{-4}	0/6	151,600
		10^{-4}	5/6	4/6	3/6	2/5	0/6	1/6	10^{-5}	4/6	15,160
147		10^{-3}	6/6	6/6	5/6	5/6	2/6	2/6	10^{-6}	4/6	1,516
		10^{-4}	6/6	6/6	6/6	5/6	4/5	4/6	10^{-7}	6/6	
73849		10^{-3}	5/6	6/6	6/6	5/6	3/6	2/6	...		
		10^{-4}	6/6	6/6	6/6	5/6	5/6	5/6			

Inaba vaccines.

Vaccine number	Challenge strain	Challenge dose	0·16	0·04	0·01	0·0025	0·000625	0·00015625	Controls		Viable count
141	V. cholerae subtype Inaba 333/52	10^{-3}	5/6	4/6	2/5	2/4	2/4	2/6	10^{-2}	0/6	18,700,000
		10^{-4}	6/6	5/6	6/6	5/6	6/6	4/6	10^{-3}	0/6	1,870,000
142		10^{-3}	6/6	5/6	6/6	3/6	5/6	0/6	10^{-4}	2/6	187,000
		10^{-4}	5/6	6/6	6/6	5/6	5/6	4/6	10^{-5}	6/5	18,700
145		10^{-3}	0/5	1/6	0/6	0/6	0/6	0/6	10^{-6}	6/6	1,870
		10^{-4}	2/6	2/6	2/6	2/6	0/5	6/6			
73850		10^{-3}	2/6	2/4	2/6	1/6	0/6	0/6			
		10^{-4}	5/6	6/6	5/6	4/6	6/6	4/6			

Numerator/denominator = Survivals/total.

From the above findings it is obvious that the serological examina of cholera vaccines yields valuable information regarding the quality suitability of the products for immunization purposes. The more elabo active and passive immunity tests do not furnish any additional infor tion which is not obtained from the serological examination alone. do not wish to decry the use of active or passive immunity tests in ass of relative potencies of cholera vaccines or in the selection of strains use as seed for vaccine manufacture, but, in our opinion, for routine assa of cholera-vaccine brews, prepared from approved smooth strains, th simple serological tests are adequate. We make this statement keeping i view the practical difficulties that many manufacturing concerns an laboratories will experience, if we insist on certain minimum requirements for anti-cholera vaccine in terms of active and passive immunity units. The use of animal tests, as a routine, would be advisable if by doing so one could obtain extra information of value which could not be got by serological tests, but frankly we are not convinced that it is so. The results of the planned collaborative assays of the vaccines sent by the Department of Biological Standardization, Copenhagen, have convinced us of the soundness of the practice which we follow at this Institute, viz. the use of serological tests only for 'passing' of finished brews of anti-cholera vaccines and the use of both serological and animal tests for selection of cholera strains to be used as seed for mass manufacture of vaccine.

Our minimum requirements at this Institute for (a) passing the vaccines as satisfactory for prophylactic purposes, and (b) for selection of a strain of *V. choleræ* to be used as seed for vaccine manufacture are as follows :—

(a) 1. The vaccine must be prepared from smooth strains. It must contain both Inaba and Ogawa sub-types in equal proportion

2. The vaccine must agglutinate with Inaba and Ogawa 'O' type specific sera.

3. The vaccine must not agglutinate with cholera rough 'O' serum.

4. In case of vaccines prepared from agar, the vibrio content must not be less than 8,000 million vibrios per c c.

(b) A smooth strain of *V choleræ* (G and V 'O' group I) isolated during the course of an epidemic must be used as the seed. It must agglu-inate to full titre with the corresponding type-specific 'O' serum The accine prepared from the strain must evoke a satisfactory vibriocidal esponse in guinea-pigs and afford protection to mice against intraperitoneal fection with a heterologous strain of *V. choleræ*. The results of active nd/or passive) immunity tests obtained must compare favourably with ose of the control vaccine maintained at the Institute as reference pre-ration for the purpose.

Our findings and comments have been communicated to the Expert nmittee on Biological Standardization of W H O and will form the basis iscussion at their next meeting of the difficult problems involved in ssay and control of cholera vaccines

II. MONO-SPECIFIC OGAWA AND INABA AGGLUTINABLE SERA.

Type-specific cholera sera prepared at the Central Research Institute, li, some four years ago and stored at the State Serum Institute, Copen-for distribution as international reference preparations were tested

TABLE II.

Passive immunity tests of cholera vaccines.

Experimental animals, Swiss mice, 17 g. to 22 g. in weight.

Immunizing dose given intraperitoneally 4 hours before challenge in a volume of 0·2 c.c.

Challenge dose given intraperitoneally in a volume of 0·5 c.c. in 5 per cent mucinized suspension.

Period of observation, 72 hours.

Ogawa vaccines.

Vaccine number	Challenge strain	Challenge dose	DOSE OF IMMUNE SERUM INJECTED (c.c.) 0·05	0·025	0·0125	0·00625	0·003125	0·0015625	Controls un-vaccinated	Viable Count
143	*V. choleræ* sub-type Ogawa strain 425/52	10^{-3}	6/6	6/6	5/6	6/6	5/6	4/6	10^{-2} 0/6 10^{-3} 1/6	10,750,000 10,75,000
144		10^{-3}	1/6	1/6	1/6	1/6	2/6	1/6	10^{-4} 3/6 10^{-5} 6/6	107,500 10,750
147		10^{-3}	6/6	6/6	6/6	5/6	4/6	3/6	10^{-6} 6/6	1,075
73849		10^{-3}	5/6	6/6	6/6	6/6	6/6	6/6		

Inaba vaccines

Vaccine number	Challenge strain	Challenge dose	0·05	0·025	0·0125	0·00625	0·003125	0·0015625	Controls un-vaccinated	Viable Count
141	*V. choleræ* sub-type Inaba strain 375/52	10^{-3}	4/6	6/6	6/6	6/6	4/6	5/6	10^{-2} 0/6 10^{-3} 1/6	16,800,000 1,680,000
142		10^{-3}	5/6	6/6	6/6	5/6	6/6	5/6	10^{-4} 5/6 10^{-5} 6/6	168,000 16,800
145		10^{-3}	4/6	3/6	1/6	1/6	1/6	1/6	10^{-6} 6/6	1,680
73850		10^{-3}	6/6	5/6	6/6	6/6	5/6	4/6		

Numerator/denominator = Survivals/total.

for type specificities and keeping qualities. No difference in titre between the samples or between the original tests carried out four years ago and the renewed tests were noticed. The sera keep up their agglutinating capacities satisfactorily under cold storage during long periods as well as during postal transmission. These sera have now been accepted as suitable international reference preparations.

III. Enzymes of *V. choleræ*.

In our report for 1952 we mentioned that an enzyme capable of dissolving collagen was present in culture filtrates of cholerogenic and non-cholerogenic vibrios. Further studies of this enzyme show that it does

The presence of elastinase in vibrio-culture filtrates has been confirmed and further work has shown that it is capable of acting both on the native and purified elastin at an optimum pH of 8. The action of this enzyme is inhibited by animal and human sera and by dialysates of ground pancreatic tissue.

The mucinolytic, tryptic and elastinolytic properties of vibrio filtrates as well as their lipase, amylase and invertase activities have been found to be due to different enzymic entities. These properties are not peculiar to *V. choleræ* alone but are exhibited by non-cholerogenic vibrios.

To assess the possible rôle of these enzymes in cholera pathogenesis, the following experiments were carried out. Membrane sacs were made using living pieces of guinea-pig ileum and the effects of vibrio filtrates on permeability of these membranes investigated. It was found that there was a notable increase in permeability when vibrio filtrates were allowed to act on the membranes. Some thermolabile constituent or constituents of the filtrates abolish peristaltic activity. The factors causing the increased intestinal permeability produce their effect independent of the muscular or nervous mechanisms concerned with peristalsis since membranes which have lost their peristaltic capacity are also equally affected by the filtrates. This activity is demonstrable when the filtrate is allowed to act on the intestine from the lumen to the peritoneum or from the peritoneal surface to the lumen. The activity is inhibited by normal serum which [illegible] as well as a trypsin inhibitor. The ultimate [illegible] of the filtrate—18 hours—on the intestinal [illegible] sues.

Histamine 1-500 in Ringer-Locke solution does not produce increase permeability or peristalsis changes when tried under identical conditions.

Filtrates of both *V. choleræ* and NAG vibrios have been observed to give identical results in permeability experiments.

Membranes consisting of the walls of blood vessels also show, like intestinal membranes, increased permeability when acted on by *V choleræ* filtrates. The effect is observed both when the action takes place on the blood-vessel wall from the periphery to the lumen or from the lumen to the periphery.

TABLE III.

Vibriocidal response to cholera vaccines.

Experimental animals — Guinea-pigs 300 g. to 400 g. in weight.
Immunizing dose—Two doses of vaccine (0·5 c.c. and 1 c.c.) at weekly intervals subcutaneously.
Vibriocidal titre tested 10 days after second immunizing dose.

Ogawa vaccines			Inaba vaccines		
Vaccine number	Vibriocidal titre		Vaccine number	Vibriocidal titre	
	Inaba	Ogawa		Inaba	Ogawa
143	1/30	1/10,935	141	1/10,935	1/10,935
144	1/405	1/135	142	1/10,935	1/10,935
147	1/3645	1/32,805	145	1/15	1/45
73,849	1/3645	1/32,805	73,850	1/32,805	1/32,805

*Method of test—As published in *Indian Journal of Medical Research*, 1948, **36**, p. 3.

TABLE IV.

Agglutination results of cholera vaccine suspensions when tested against mono-specific cholera 'O' sera and cholera rough 'O' sera.

Ogawa vaccines				Inaba vaccines			
Vaccine number	Inaba type 1000	Ogawa type 1000	Cholera rough 1000	Vaccine number	Inaba type 1000	Ogawa type 1000	Cholera rough 1000
143	—	1000	—	141	500	—	—
144	—	500	500	142	500	—	—
147	—	500	—	145	50	50	500
73,849	—	250	—	73,850	500	—	—

2. Investigation on cholera endemicity with special reference to Hilsa and other estuarine fishes under Dr. M. N. Lahiri, at the All-India Institute of Hygiene & Public Health, Calcutta.

Collection of water samples was continued from the surface and bottom of a selected stretch of the river Hooghly, both from the shore and the mid-stream, at three fishing centres, viz. Nawabgunge, Baghbazar and Falta or Diamond Harbour. The last station which is an important endemic centre for cholera was selected when fishing was closed at Falta. A total of 722 samples of water were examined, and out of these, three samples were positive for cholera vibrios and 306 samples for NAG vibrios.

*Ahuja, M.L., and Gurkirpal Singh (1948), Observations on cholera vaccines-*Ind. Jour. Med. Res.* **36**, 1, p. 3.

For the corresponding period last year, seven positive isolations of cholera vibrios were made. All these isolations were obtained from March to May, but the period of occurrence this year was found to extend up to July from March

As during the previous year, all the isolations were made from the upper reaches of the estuary (Nawabgunge) at high tide only. Two isolations were made from the surface and one from the bottom. Out of the three strains isolated, two were of the Ogawa type and one of the Inaba type. NAG vibrios isolated from water samples were grouped according to Heiberg's classification. Groups I, II and V formed the predominant constituents of the vibrio flora of water The seasonal fluctuations of groups I and II show a definite correlation with the period of cholera epidemics in Calcutta.

The pH and salinity of the water samples collected from the three fishing centres were determined with a view to elucidate any probable correlation between these values and the occurrence of vibrios. While the fluctuations in pH are not very marked, the salinity varies to an appreciable extent. Besides the diurnal variations due to tidal influence, seasonal variations are caused by the large quantities of flood water brought down during the S.-W. monsoons and the excessive evaporation during the summer months. As is to be expected, the average salinity is higher at the lower reaches of the estuary at Falta and Diamond Harbour than in the upper reaches at Nawabgunge and Baghbazar. It is interesting to note that isolations of cholera vibrios have been made only from the upper reaches of the estuary. No definite correlation appears to exist between the occurrence of vibrios and the salinity fluctuations of the Hooghly water.

The possibility of vibrios being adsorbed by the silt particles in the water and being deposited on the estuarine bottom, where they thrive, was considered to be a fruitful line of investigation. Pending the development of a technique for the concentration of bacterial flora from a large quantity of silt, the top l [illegible] 1 sq. ft. was collected by hand, fr [illegible] which about 10 g. were taken at [illegible] No *Vibrio choleræ* have so far been isolated, but NAG vibrios have been obtained. Out of a total of 102 samples examined, 44 were positive for vibrios No distinct relationship between the seasonal fluctuations in the occurrence of the two predominant groups, viz. groups I and II, and the seasons of cholera epidemics could be traced from the available data.

Preliminary experiments were conducted to study the survival of vibrios in silt. Silt collected from the Hooghly river bed was sterilized and crushed into a fine powder. In test-tubes containing 5 g of this silt, 10 c c. of water were added and live cultures of AG and NAG vibrios were inoculated into them, keeping suitable controls. Significant differences were not found in the survival of vibrios in the water and the silt, but it is necessary to repeat the experiments on a larger scale to arrive at any definite conclusions.

Different size groups of hilsa ranging from the fry stage to the adult stage were collected during the seasons of their availability in the river Hooghly from the three selected centres every week and their gut-contents and gill-samples bacteriologically examined. During the off-season for Hilsa, other estuarine fishes available were examined. No *V[illegible] choleræ* have so far been isolated, but NAG vibrios were [illegible] of 204

samples of Hilsa, consisting of 648 specimens and 55 samples of other fishes consisting of 268 specimens, the gut-contents of 76 samples of Hilsa and 34 samples of other fishes were positive. The gills of 77 samples of Hilsa and 26 samples of other fishes were also positive for vibrios. NAG vibrios have been found to occur in the fishes, either in the guts or the gills, throughout the year. Groups I, II and V are the three predominant groups of NAG isolated from the alimentary tracts of fishes. The seasonal fluctuations in the occurrence of groups I and II show a definite correlation with the periodicity of cholera in Calcutta. Parallel data for the gill samples also give a picture somewhat similar to the one obtained for the gut-contents. In view of the above relationship it seems that groups I and II vibrios are in some way connected with the maintenance of cholera endemicity.

In the absence of better equipment, the pH of the gut-contents of Hilsa was studied by the spot-test method. The stomach-contents were found to be definitely acidic and the intestinal contents showed a pH ranging between 6 and 7. In view of the possibility that the gut-contents may be undergoing some chemical changes during the period that elapses between the capture of the fish and their dissection in the Laboratory, thus affecting the viability of the vibrios, similar samples of fish were dissected in the field and in the Laboratory. No significant differences were noticed in the pH or the vibrio flora of these two sets of samples.

As a preliminary to artificial infection experiments envisaged in the programme, it was necessary to perfect a method of collection, conditioning and rearing of Hilsa in confined areas. Hilsa is an extremely delicate fish and it was believed that it will die immediately after it strikes the net used for its capture. The settling tanks and filter-beds of the Calcutta Corporation Water Works at Pulta was considered the cleanest and most suitable source from which collections could be attempted. After a good deal of experimentation, it was possible to perfect a method of collecting young Hilsa and rearing them in cement cisterns $10' \times 3' \times 3'$. Water from the settling tanks was fed into these cisterns and plankton was provided as fish food.

In view of the possible difficulties in transporting the fish to Calcutta for the artificial infection experiments, it was decided to conduct these in Pulta in cement cisterns at the premises of the Central Inland Fisheries Research Station. Before these experiments are undertaken, it was, however, thought advisable to try some small-scale experiments with hardier fishes in the Laboratory in the form of pilot experiments. Kôi (*Anabas testudineus*) and Lata (*Ophicephalus striatus*) were employed for these studies.

Specimens of these two species were reared separately in vibrio-free water in glass aquaria and fed on vibrio-free food. The water was renewed daily and the old water examined bacteriologically for the presence of vibrios. When the water proved negative for three consecutive days, they were fed on infected food. Pupæ and larvæ of house-flies bred in the Laboratory and fed on vibrio-free milk were injected with live cultures of cholera vibrios and the fish were fed on these. Every day, the fish were removed to fresh sterilized aquaria, after washing them several times in vibrio-free water. The water from the aquaria was examined daily, but no vibrios were recovered for over 30 days. The fish were then dissected and their intestinal contents bacteriologically examined. Hæmolytic non-agglutinable vibrios were recovered.

This result led to an examination of the possibility of the vibrios remaining in the guts without being excreted, as also of the artificially infected specimens having had vibrios in their alimentary tracts before they were infected. After a consideration of possible means of checking up the vibrio-free nature of the gut of a fish before it is artificially infected, it was decided to conduct an experiment to determine this.

A sample of thirty Kôi (*Anabas testudineus*) was reared in a large tub in vibrio-free water which was changed every day. The fish were fed on boiled yolk of eggs regularly. Samples of 1,000 c.c. of water were examined from the tub daily for the presence of vibrios and after they were negative consecutively for over a week, ten specimens were taken at random and dissected to find out if vibrios were present in their guts. One fish showed the presence of inagglutinable vibrios. The experiment is now being repeated with smaller number of fish and lesser quantity of water, so that the whole water in the aquaria can be examined bacteriologically and the possibility of vibrios having remained undetected can be ascertained.

Particular attention was directed to the study of the cholera enzyme, mucinase present in the filtrates of *V. choleræ* which has been reported to cause sloughing of the mucosa from strips of guinea-pigs ileum (Burnet & Stone, 1947). After a good deal of experimentation a method for the quantitative titration of the enzyme has been standardized. It has been observed that mucinase elaboration and the maintenance of quantitative activity are influenced by several factors, i.e. media, pH, moisture, etc.

Twenty-seven strains of *V. choleræ* (21 Ogawa sub-type and 6 Inaba sub-type) isolated from cholera patients and from Hooghly river water during the past two years were tested. With each strain the mucinolytic activity was evident at a titre of 1 in 1,600 or above. One strain, however, showed a very high titre of 1 in 25,600, and a second strain tested on four occasions during a period of six months gave a constant titre of 1 in 6,400. The presence of this enzyme has also been demonstrated in certain intestinal organisms, i.e. *B. coli* type I, *Salm. enteritidis*, and *Sh. flexneri*, but the titre was of a very low order and never exceeded 1 in 10. In the course of this work a smooth strain of *V. choleræ* became rough, and when tested later its mucinase-forming property was found to be very considerably lowered. It is presumed that this is true for all strains of *V. choleræ*, if or when they become rough. Subsequently, investigations were carried out with 18 strains of NAG vibrios. These were isolated from Hooghly [illegible]

variation in the elaboration of the enzyme but the titre was low and ranged from 1 in 100 to 1 in 400. Thus, it appears that the intensity of mucinase activity is of greater importance rather than its mere presence.

Since high titres have so far been obtained with true cholera vibrios, it appears that it may be possible to utilize the test as an adjunct to other known tests which characterize such vibrios.

MALARIA.

Insecticide and mosquito repellent inquiry under the Director, Malaria Institute of India, Delhi.

The following investigations were carried out in the Laboratory and field :—

A. Laboratory investigations :

1. Solubility of DDT crystals in cuticular wax of house-flies and mosquitoes.

2. Resistant strain of *Culex fatigans.*

3. Susceptibility of DDT-resistant strain of *Culex fatigans* to other chlorinated hydrocarbon insecticides.

4. Effect of feeding normal and resistant strains of *Culex fatigans* on DDT-fed fowls.

5. Rapid method of screening DDT water-dispersible powder samples.

B. Field investigations :

1. Studies on the behaviour of mosquitoes in relation to insecticidal application.

2. Evaluation of DDT, BHC, DDT and BHC combined and dieldrin residual sprays against mosquitoes in different parts of India.

C. Confirmation of findings of the insecticide and mosquito repellent inquiry.

D. Publications.

A. Laboratory investigations.

1. *Solubility of DDT crystals in cuticular wax of house-flies and mosquitoes*—Pal, (*Bull. Ent. Res.*, **41**, 1, pp. 121-139, 1950) studied the wetting of insect cuticle by insecticidal liquids ; as the effectiveness of almost all contact insecticides depends upon it. He found that apart from the chemical and physical nature of the cuticular lipoids, irregularities on the body surface were important. With the application of residual deposits of insecticides, however, the position is completely different. The insecticide is not available in the form of liquid but as crystals. Under such circumstances the rate and degree of dissolution of crystals in the cuticular wax is an important factor affecting the toxicity of contact insecticides. The object of the present investigation was to study the solubility of DDT in the cuticular wax of insects particularly those of public health importance.

Cuticular wax of *Musca nebulo*, *Anopheles stephensi* and *Culex fatigans* were extracted in chloroform by soaking a large number of insects in sufficient quantity of chloroform for 24 hours. The lipoid-chloroform solution was filtered through a cintered glass-funnel and the chloroform was allowed to evaporate at room temperature. One c.c. of 1 per cent solution of cuticular wax in chloroform was uniformly applied on the excavated portion of a cavity glass-slide (area about 2·27 sq. cm.). Technical DDT specially pulverized was dusted on the wax-treated area of the slide after ensuring complete evaporation of chloroform.

Micro-photographs of the crystals on wax were taken without disturbing the slide, after 5 and 30 minutes, 1, 3, 6 and 24 hours.

It was observed that the dissolution of crystals in the cuticular wax of *Musca nebulo* and *Anopheles stephensi* started almost immediately and they almost completely disappeared in 3 and 6 hours, respectively. In the case of *Culex fatigans* crystals were observed intact even after 24 hours. This is of considerable interest because these observations are in conformity with the natural biological resistance of *Culex fatigans* to DDT residual deposits.

2. *Resistant strain of* Culex fatigans.—Reports of culicine mosquitoes having developed resistance to DDT have been received from several parts of the wold. In those states of India where DDT residual spraying has been in progress for the last six years or so, there is a possibility of certain culicine species having developed resistance to DDT. Investigations were undertaken in Delhi State where DDT has been in use since 1946. *Culex fatigans* were captured from a sprayed village Khurauji Khas which had been regularly sprayed with DDT during the past six years, and from an unsprayed village Ram Garhi (U.P.) 10 miles apart. These two strains were reared for one generation simultaneously under identical conditions. [illegible] The mosquitoes [illegible] h) coated with [illegible] per sq. ft. The [illegible] Cent per cent knock-down of mosquitoes collected from unsprayed village was obtained within five hours, whereas only 66 per cent (females) and 79 per cent (males) mosquitoes from sprayed village were knocked-down during that period. Even after 24 hours contact, only 78 per cent (females) and 24 per cent (males) mosquitoes were knocked-down. In subsequent tests the mosquitoes were exposed for half-an-hour to glass-panels sprayed with DDT at the rate of 50 mg./sq. ft. The exposed insects were kept under observation for 48 hours. Twenty experiments were carried out, mortality amongst the mosquitoes from sprayed village was nine per cent (females) and 31 per cent (males) after 48 hours, and the corresponding figures for the mosquitoes from unsprayed village were 90 and 93 per cent, respectively.

3. *Susceptibility of DDT-resistant strain of* Culex fatigans *to other chlorinated hydrocarbon insecticides*.—One object of this investigation was to determine whether or not DDT-resistant strain of *Culex fatigans* was also resistant to other chlorinated hydrocarbon insecticides. Both normal and resistant strains were exposed to glass-panels treated with DDT, chlordane, dieldrin applied at the rate of 50 mg./sq. ft. and BHC gamma isomer at the rate of 10 mg /sq ft. for 5 and 15 minutes, and were then kept under observation in clean cages for 24 hours after which the mortality was recorded. It was found that the mortality rate was comparatively low when the resistant strain was exposed to DDT, whereas in the case of other insecticides the mortality rates were high and almost identical to those of the normal strain. It seems that the DDT-resistant strain of *Culex fatigans* is initially not cross resistant to other insecticides tested.

[illegible]

(*ii*) Since 1950, in the field experiments with different insecticides against mosquitoes, total mosquito-catch data from sprayed and unsprayed catching stations of the treated villages was found very useful in evaluating residual sprays (Pal, R. *Ind. J. Mal.*, **5,** pp. 195-199, 1951. Entomological observations from comparison village may sometime vary from the experimental villages due to some natural causes and hence are not always helpful in the assessment of results. Other workers (Rao, B. A. 1953, *personal communication*) have also expressed similar opinion and they also consider that entomological data from a comparison village is unnecessary ; though it may provide useful epidemiological data.

(*iii*) Resistance in terms of knock-down or mortality; Pal and Sharma (1951, *Proc. IXth Internat. Cong. Entomol.*) and Pal *et al.* (*Ind. Jour. Malariol.*, **6,** pp. 303-316 1952), in their studies on the development of resistance to insecticides in housflies concluded that resistance of flies should be specifically mentioned in terms of 'knock-down resistance' and 'mortality resistance' because wide variations exist between the two. These findings have been confirmed by Busvine, J. R. (*Nature,* **171,** pp. 118-119, 1953).

D. Publications.

2. Inquiry to study nutritional states and their effects on mammalian malaria under the Director, Malaria Institute of India, Delhi.

The object of the inquiry is to determine the effect of different nutritional states of the host (rats) on the host-parasite relationships of animals with *P. berghei* infections.

It has been stated in the last year's report that multiplication of this parasite in starved host was not of the order as in normally fed hosts. Three possible reasons were considered, namely an increased resistance in the host to invasion of parasites, starvation of parasites themselves and ketosis in the host. The first possibility was ruled out as the parasites multiplied normally in infected hosts which were fed after a certain period of starvation. The second possibility was perhaps the most probable cause of infection not establishing itself during starvation. Infected animals were fed on PABA during starvation and the parasites became patent though their density was low. This seemed to show that PABA is probably one of the essential nutrilites for *P. berghei* and that it is not readily available in sufficient quantity to the parasites during starvation of the host. Similar experiments during the current year showed that methionine is another essential nutrilite not available during starvation.

Thirdly, a highly ketogenic diet consisting of a high proportion of butter-fat was given to infected animals. The parasitæmia was less than in controls fed on normal diet and showed the probability of ketosis being an additional factor for the restricted parasitic growth in starved hosts.

The age of the animal appears to be important in the consideration of its host-parasite relationship during its starvation. In two experiments it was found that when six-week old infected animals were starved, the parasites were patent in considerably higher numbers and for a longer period than in similarly treated adult animals. The high susceptibility of young animals to infection seems to persist even when they are starved.

Feeding infected and starved hosts on chemically pure nutrilites, either singly or in combination, has led to a better understanding of the

physiological needs of parasites. The scope is wide in respect of vitamins, particularly with the availability of specific vitamin antagonists.

Effect of different quantities of the same diet on the course of infection in albino rats

Six replicate experiments were carried out in 108 rats. The experimental animals were given half the quantity of standard diet for control animals.

Under-nourishment, in so far as malaria is concerned, bears a two-fold effect: one on the malaria parasites and the other on the host's defence mechanism. The parasite in the under-nourished host grows and multiplies to a less extent than in a well-nourished host. An interesting feature is that due to impaired capacity of the host to develop immunity even mild infection tends to end fatally.

Under-nourishment seems to greatly affect the capacity of the host to acquire immunity. If an infected under-nourished (or starved) host is fed normally the parasite reaps the benefit more quickly than the development of host-defence mechanism. Under such circumstances, the parasite multiplies rapidly both due to better available nourishment as well as the absence of adequate check by host immunity. More often than not the host dies. Such a sequence of events probably explains the high case mortality during malaria epidemics and in particular the one which occurred during the Bengal famine of 1943 It was noticed that institution of relief measures, at a time when fresh transmission was low or absent, resulted in greater number of deaths due to malaria.

Effect of different quality diets on the course of infection in rats

One hundred and fifty rats were used for the study which consisted of six experiments, some of which were replicates. The animals in each group, experimental as well as control, were mostly of the same age and sex distribution.

The different diets used in the experiments were of three types representing vegetarian, lacto-vegetarian and mixed. It was ensured that these diets were not deficient in any of the vitamins or minerals.

Vegetarian diets of a high-carbohydrate content, containing rice or wheat gave, rise to a more severe acute infection than normal balanced diet. Very few of the animals on such a diet died of chronic infection and the degree of acquired immunity was sufficient to nullify a challenge inoculation. Lacto-vegetarian diets consisting of milk and rice or wheat were responsible for a milder acute infection as compared to vegetarian diet, though the course of chronic infection was similar in animals on either of the above diets.

Animals on a diet with a high proportion of meat suffered from a more severe, acute as well as chronic, infection than those on other diets. It seemed logical that it sould be so in view of the fact that such a diet probably provided, in abundance, all nutrients required by the parasite, far in excess of its capacity to enhance the acquirement of immunity by the host.

Among the different proteins constituting these diets, milk protein more clearly established a host-parasite relationship in favour of the host.

alone is the method of choice and in this inquiry attempts have been made to make the spraying of the test panels conform in a large measure to what is obtained in malaria control operations in the field, without at the same time losing sight of the required necessities for laboratory spraying, such as capacity to deliver equal doses repeatedly and an assurance that all parts of the test panels are fairly uniformly covered. The use of an ordinary Hudson pneumatic sprayer with a fan-shaped nozzle make the equipment very near to conditions of spraying.

In the spraying equipment devised for spraying the test panels, an ordinary Hudson pressure sprayer is used with a flat-spray nozzle. The test panels move on canvas belt running over two metal rollers driven by an electric motor. The insecticide is discharged at a pressure of 30-lb. to 28-lb. at which pressure the spraying of the panels is done and the pressure is not allowed to fall below 28 lb. The nozzle is adjusted so that the width of the spray just touches the edges of the panel and the entire surface is thus assured of a uniform spray.

Method of working.

(1) The motor is switched on and the number of revolutions done by the canvas belt in a second is calculated.

(2) The discharge rate of the pump per second is calculated at 30-lb. to 28-lb. pressure.

(3) The time taken for the belt to travel a distance equal to the length of the panel is calculated.

(4) The strength of the spraying fluid is adjusted to see that the required dosage is sprayed over the panel.

In this inquiry panels had to be spayed in two dosages:—

(i) 50 mg. per square foot.

(ii) 200 mg. per square foot.

This meant that the panels which measured 6″×5·7″ have to be sprayed so as to get 11·8 mg. of DDT for the lower dosage or 47·2 mg. of DDT for the higher dosage.

The time taken for the panel to travel its own length was found to be 0·5 second.

In one second the sprayer, at 30-lb. to 28-lb. pressure, discharged 20 c.c. Therefore, in 0·5 second, the total discharge will be 10 c.c.

Now, this 10 c.c. must be made to contain the 11·8 mg. or 47·27 mg. of DDT as the case may, be so that the DDT may be uniformly sprayed over the test panel. In this strength alone, 5,000 c.c. have to be prepared so that the pneumatic sprayer may work satisfactorily.

A large zinc tray to contain the pneumatic sprayer and the moving belt was found useful in collecting the spilt DDT emulsion or suspension at the time of the spray.

Test insects.

Test insects were being reared under comparative conditions with the same quantity of food supply and water. The pupæ that develop on any one day have been utilized for the test. *A. stephensi* type has been used for all the tests recorded in this inquiry.

STUDY OF SOIL TYPE.

The Madras State is a part of the land surface of Indian peninsula, which has remained stable and undisturbed for untold geological ages The parent rock of the geological formation is responsible for the variable soils present, as for instance the difference between the black and red soils or if they are of alluvial origin on the rock formation in the catchment area of the river bringing them down and depositing them in the deltas

The soil—The soil as ordinarily conceived consists of the following :—

(*a*) Mineral materials.

(*b*) Organic matter.

(*c*) Water.

(*d*) Air.

These, in the main, are in a fine state of subdivision and are intimately mixed. In fact, the contact is often so close as to render satisfactory separation rather difficult.

The mineral material has its genesis in the regolith or soil material. Some of the soil minerals have persisted more or less unchanged, while others have developed as the regolith weathered either before or during soil formation. Naturally, various sizes of particles occur ranging from those which are coarse, such as gravel and sand, to those, such as silt and clay which are in a fine state of division.

The organic matter represent the accumulation of plant and animal residues and is generally in an active state of decay.

An examination of any soil will reveal the presence of pore spaces of varying sizes. These occur not only between the large solid particles, but also between and within the clumps and aggregates which the fine particles tend to form. The pore spaces variable as to continuity, dimensions and total volume are occupied in large part by water and air, the proportion depending on the character of the soil and the conditions under which it is functioning.

Water, the third component of the soil is held within these pore spaces with varying tenacity due to certain surface forces. Soil water also carries many soluble salts.

The principal gases of the soil air, the fourth component, are nitrogen, oxygen and carbon dioxide

Volume and composition.—A silt-loam surface soil contains approximately 50 per cent of solids and 50 per cent of pore space. This pore space will be somewhat less for sandy soils and somewhat more for soils of a more clayey nature. The 50 per cent of solid space is occupied by about 45 per cent of mineral and 5 per cen[illegible] . The proportion of air and water is, of [illegible] under natural conditions depending on [illegible]

In short, the soil may be visualized as possessing a frame work of mineral matter—in part non-colloidal and inert ; in part colloidal and extremely active . In mineral soils clayey materials make up much of this latter fraction, but mixed with this viscous, galatinous mineral[illegible]

partially adsorbed thereby, is a certain amount of humus, notably colloidal and unstable clay and humus, more or less intimately mixed constitute most of the colloidal matter found in soils. This finely divided fraction not only promotes activity catalytically, but may also participate intensively in the changes that occur. In fact the latter may react and be reacted upon to the extent of losing its colloidal character in the production of simple compounds. Adsorption, plasticity, cohesion and certain chemical changes are all controlled by substances in the colloidal state. While humic colliods are the more active, the presence usually of a larger amount of viscous, gelatinous and more stable clayey matter preserves a balance in their relative importance.

In Agriculture five soil types referred to below are recognized in the Madras State :—

1. The red soils.
2. The black soils.
3. Coastal alluvium.
4. Delta soils.
5. Laterite soils.

Types 1, 3 and 5 are predominantly red, reddish or brown in colour, while types 2 and 4 are dark coloured popularly described as black. Type 1 occurs over a large part of the Madras State and may be said to be the common type. It occurs in all sorts of situations ranging from hill slopes to deep valleys between hills in various parts of the State. Types 3 and 5 are also red or reddish brown, though they form different soil types from the point of view of the soil chemist.

Type 2 is a characteristic soil occupying, for the most part, the central plateau of the peninsula.

Type 4, the delta soil, occurs mainly on the deltas of the rivers.

The laterite type 5 occurs in the West Coast and in some parts of the East Coast.

To start with, samples were obtained from six areas of the Madras State and the chemical analysis of the soils is furnished in Table I :—

TABLE I.

Chemical analysis of samples of earth.

Heads of analysis.	Araku, per cent.	Chaga-nur, per cent.	Ennore. per cent.	Dhim-bam, per cent.	Mala-bar, per cent.	Tonjore per cent.
(*a*) *Chemical analysis* :						
Moisture	0·30	6·02	3·01	4·36	2·82	0·82
Less in ignition ...	23·95	6·53	1·81	11·61	4·43	3·05
Iron oxide (Fe,2°3) ...	23·84	3·41	1·96	6·17	4·60	2·18
Alumina (A12°3) ...	46·73	7·60	3·95	12·87	8·71	7·43
Insolubles ...	5·6	8·5	91·14	68·74	81·98	87·08
(*b*) *Mechanical analysis* :						
Clay	10·53	47·13	19·45	38·63	21·35	27·33
Silt	8·88	14·10	1·10	11·55	9·22	2·57
Fine sand ...	8·92	18·06	3·06	14·01	21·06	15·24
Coarse sand ...	68·92	15·86	75·60	33·48	48·34	56·61
Acid solubles ...	2·75	4·81	0·79	2·33	0·03	...
TOTALS ...	100·00	100·00	100·00	100·00	100·00	101·75